

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6: <b>C12N 15/12, C07K 2/00, 4/12, 14/435, A61K 38/17</b>		A1	(11) International Publication Number: <b>WO 97/04091</b> (43) International Publication Date: <b>6 February 1997 (06.02.97)</b>
(21) International Application Number: <b>PCT/AU96/00460</b> (22) International Filing Date: <b>19 July 1996 (19.07.96)</b>		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(30) Priority Data: PN 4263 20 July 1995 (20.07.95) AU PN 6847 27 November 1995 (27.11.95) AU PN 7299 22 December 1995 (22.12.95) AU PN 7890 5 February 1996 (05.02.96) AU		(71) Applicant (for all designated States except US): <b>AMRAD OPERATIONS PTY. LTD. [AU/AU]; 17-27 Cotham Road, Kew, VIC 3101 (AU).</b> Published With international search report.	
(72) Inventor; and (75) Inventor/Applicant (for US only): <b>NICOLA, Nicos, Anthony [AU/AU]; 56 Churchill Street, Mont Albert, VIC 3127 (AU).</b>			
(74) Agents: <b>HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).</b>			
(54) Title: <b>NOVEL RECEPTOR LIGANDS AND GENETIC SEQUENCES ENCODING SAME</b>			
(57) Abstract			
<p>The present invention relates generally to novel receptor ligands and to genetic sequences encoding same. The novel receptor ligands and their encoding genetic sequences are useful in the development of a wide range of agonist, antagonist, therapeutic and diagnostic reagents based on ligand-receptor interaction. The present invention further provides a means for identifying ligands without knowledge of their receptor and a means for identifying receptors.</p>			

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LJ	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

- 1 -

**NOVEL RECEPTOR LIGANDS AND GENETIC SEQUENCES  
ENCODING SAME**

5 The present invention relates generally to novel receptor ligands and to genetic sequences encoding same. The novel receptor ligands and their encoding genetic sequences are useful in the development of a wide range of agonist, antagonist, therapeutic and diagnostic reagents based on ligand-receptor interaction. The present invention further provides a means for identifying ligands without knowledge of their receptor and a means for identifying receptors.

10

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined at the end of the description.

Throughout this specification, unless the context requires otherwise, the word "comprise", or 15 variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The rapidly increasing sophistication of recombinant DNA technology is greatly facilitating 20 research into the medical and allied health fields. Research into cellular growth factors such as cytokines is of particular importance due to their involvement in proliferation, differentiation and function of a wide variety of cells. Administration of recombinant growth factors or of molecules capable of regulating growth factor function and/or synthesis is becoming increasingly the focus of medical research into the treatment of a range of conditions.

25

Many cellular growth factors and other extracellular mediators of cell function exert their actions by interacting with cell surface receptor protein tyrosine kinases (RPTKs). Ligand binding to such receptors usually results in receptor aggregation, cross-phosphorylation on tyrosine residues of the receptor cytoplasmic domains, activation of the intrinsic kinase activity 30 of the cytoplasmic domains and phosphorylation of down-stream cell signalling molecules

- 2 -

(Schlessinger and Ullrich *Neuron* 9: 383-391, 1990; Flanti *et al* *Ann. Rev. Biochem.* 61: 453-481, 1993; van der Greer and Hunter *Ann. Rev. Cell. Biol.* 10: 251-337, 1994).

RPTKs can be subdivided into several classes dependent primarily on the predicted structural features of their extracellular domains and whether or not they contain an uninterrupted tyrosine kinase domain in the cytoplasmic portion of the receptor (Hilton In "Guidebook to Cytokines and Their Receptors" N.A. Nicola ed, Oxford University Press, Oxford pp 8-16, 1994). The largest class of RPTKs is known as the eph-related kinases (ERKs) and include the tyrosine kinases eph, elk, cck, eek, erk, cek 4-10, mek 4, hek, tk2, tyro 5 and sek. They are characterised by an extracellular sequence that includes a single cysteine-rich repeat domain followed by two fibronectin type III domains and an uninterrupted tyrosine kinase domain in the cytoplasmic region.

Despite the fact that ERKs comprise the largest subclass of RPTKs, most of the ERKs were identified without prior knowledge of their biological function nor of the ligands with which they interact. Nevertheless, the expression patterns of the ERKs suggest that they might have important roles in early vertebrate development and in brain function and hemopoietic cell function as well as functions on other organs in the adult (Cheng and Flanagan *Cell* 79: 157-168, 1994; Shao *et al* *J. Biol. Chem.* 270: 3467-3470, 1995; Beckman *et al* *EMBO J.* 13: 3757-3762, 1994).

There is clearly a need, therefore, to identify and characterise ERKs. There is also an important need to identify ligands for ERKs with or without prior knowledge of the specific ERK to which a ligand might interact.

25

Recently, some ligands that can bind to ERKs have been identified and are referred to as "ligands for ERKs" (LERKs) amongst other names. The first of these, LERK1, is equivalent to the B61 gene product previously identified as an interleukin-1 - or tumour necrosis factor-inducible product of human umbilical vein endothelial cells (Holzman *et al* *Mol. Biol.* 10: 5830-5838, 1990; Beckmann *et al* *EMBO J.* 13: 3757-3762, 1994). LERK2 appears to be

- 3 -

identical to human ELK ligand (ELK-L) and the human homologue of the mouse proteins elg 2 (eph-ligand 2) and cek5 L (cek5-ligand) (Beckmann *et al* *EMBO J.* 13: 3757-3762, 1994; Fletcher *et al* *Genomics* 24: 127-132, 1994; Shao *et al* *J. Biol. Chem.* 269: 26602-26609, 1994; Davis *et al* *Science* 266: 816-819, 1994). LERK3 is nearly identical to EHK1-L (ehk1-5 ligand) Kozlosky *et al* *Oncogene* 10: 299-306, 1995; Davis *et al* *Science* 266: 816-819, 1994), LERK4 is unique (Kozlosky *et al* *Oncogene* 10: 299-306, 1995) and the cek7 ligand is identical to elf-1 (eph ligand family-1) (Shao *et al* *J. Biol. Chem.* 270: 3467-3470, 1995); Cheng and Flanagan *Cell* 79: 157-168, 1994). All of these ligands appear to be cell surface-associated either through a C-terminal glycosyl-phosphatidyl inositol (GPI)-linkage (LERKs 10 1, 3 and 4) or through a traditional transmembrane domain (LERK2). Most appear to require cell surface expression or soluble dimer forms in order to activate the tyrosine kinase activity of the appropriate ERKs (Davis *et al* *Science* 266: 816-819, 1994). Moreover, none of the LERKs described so far show an absolute specificity for one ERK. For example, LERK1 binds to hek, elk and eck; LERKs 2, 3 and 4 bind to hek and elk and cek 7-L binds to sek and mek 15 4 with equilibrium dissociation constants varying from 1 nM to 500 nM.

In accordance with the present invention, the inventors have identified a new LERK which will assist in the development of a range of new therapeutics and diagnostics and in the identification of new ERKs.

20

Accordingly, one aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form encoding at least one of the following amino acid sequences:

- 25 (i) VXWXSXN [SEQ ID NO:1];  
(ii) DXXDIXCP [SEQ ID NO:2];  
(iii) EXYXLYXVXXXXXXXXC [SEQ ID NO:3]; and/or  
(iv) KFQXXXXXXXXGXEFXXXHXYXXI [SEQ ID NO:4];

wherein X is any amino acid residue.

30

- 4 -

Preferably, the nucleotide sequence or its complementary form encodes a product comprising at least two of, more preferably at least three of and even more preferably all four of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4.

5 In this regard, the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form encoding an amino acid sequence selected from the list consisting of:

- 10 (i) VXWXSXN [SEQ ID NO:1];  
(ii) DXXDIXCP [SEQ ID NO:2];  
(iii) EXYXL YXVXXXXXXXXC [SEQ ID NO:3]; and  
(iv) KFQXXXXXXXXXGXFXXXHXXXXI [SEQ ID NO:4],

wherein X is any amino acid residue.

15

Preferably, the nucleic acid molecule of the present invention encodes a LERK referred to herein as "NLERK2", said nucleic acid molecule selected from the list consisting of:

- (i) a nucleic acid molecule comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:5;  
20 (ii) a nucleic acid molecule comprising a sequence of nucleotides having at least about 70% similarity to the nucleotide sequence set forth in SEQ ID NO:5; and  
(iii) a nucleic acid molecule capable of hybridising under low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:5.

- 25 The nucleotide molecule is preferably derivable from the human genome but genomes and nucleotide sequences from non-human animals are also encompassed by the present invention. Non-human animals contemplated by the present invention include livestock animals (e.g. sheep, cows, pigs, goats, horses, donkeys), laboratory test animals (e.g. mice, rats, guinea pigs, hamsters, rabbits), domestic companion animals (e.g. dogs, cats), birds (e.g. chickens, geese, 30 ducks and other poultry birds, game birds, emus, ostriches) and captive wild or tamed animals

- 5 -

(e.g. foxes, kangaroos, dingoes).

Preferred human genomic sequences include sequences from brain, liver, kidney, neonatal, embryonic, cancer or tumour-derived tissues.

5

Once a novel nucleotide sequence is obtained as indicated above encoding a LERK, oligonucleotides may be designed which bind cDNA clones with high stringency. Direct colony hybridisation may be employed or PCR amplification may be used. The use of oligonucleotide primers which bind under conditions of high stringency ensures rapid cloning 10 of a molecule encoding the novel LERK and less time is required in screening out cloning artefacts. However, depending on the primers used, low or medium stringency conditions may also be employed.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% 15 v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at 20 least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

25 Accordingly, another aspect of the present invention contemplates a method for cloning a nucleotide sequence encoding a novel LERK, said method comprising searching a nucleotide data base for a sequence which encodes at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4, designing one or more oligonucleotide primers based on a nucleotide sequence located in the search, screening a nucleic acid library with said one or more oligonucleotides 30 and obtaining a clone therefrom which encodes said novel LERK or part thereof.

- 6 -

Preferably, the nucleic acid library is a cDNA, genomic or mRNA library.

Preferably, the nucleic acid library is a cDNA expression library.

5 Preferably, the nucleotide data base is of human origin such as from brain, liver, kidney, neonatal tissue, embryonic tissue, tumour or cancer tissue.

In one preferred embodiment, the oligonucleotide primers are selected from SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID

10 NO:13.

In a particularly preferred embodiment the LERK is selected from the list consisting of:

- (i) a nucleotide sequence substantially as set forth in SEQ ID NO:5;  
15 (ii) a nucleotide sequence having at least about 75% similarity to the nucleotide sequence set forth in SEQ ID NO:5; and  
(iii) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence as set forth in SEQ ID NO:5.

In an alternative aspect of this embodiment, medium stringency conditions are employed. In 20 another alternative aspect, high stringency conditions are employed.

Another aspect of the invention is directed to the genomic gene and to 3' and 5' regions thereof.

The 3' region of the NLERK2 gene is represented in SEQ ID NO:14 and the 5' region is shown in SEQ ID NO:15.

25

Accordingly, another aspect of the present invention is directed to a nucleic acid molecule corresponding to or derived from the 3' region of the genomic gene encoding NLERK2 or its derivatives, said nucleic acid molecule or its complementary form having a nucleotide sequence substantially as set forth in SEQ ID NO:14 or having at least 75% similarity thereto or capable 30 of hybridising thereto under low stringency conditions. Other levels of stringency such as

- 7 -

medium and high may also be used.

A further embodiment is directed to a nucleic acid molecule corresponding to or derived from the 5' region of the genomic gene encoding NLERK2 or its derivatives, said nucleic acid 5 molecule or its complementary form having a nucleotide sequence substantially as set forth in SEQ ID NO:15 or having at least 75% similarity thereto or capable of hybridising thereto under low stringency conditions. Other levels of stringency such as medium and high may also be used.

- 10 The present invention also extends to hybrid genetic sequences comprising a coding region of a structural gene flanked or fused to one or both of SEQ ID NO:14 and/or SEQ ID NO:15 or derivatives thereof.

Still another embodiment contemplates the promoter or a functional part thereof of the genomic 15 gene encoding NLERK2. The promoter may readily be obtained by, for example, "chromosome walking".

Another aspect of the present invention is directed to NLERK2 including a recombinant polypeptide having an ability to interact with an ERK wherein said NLERK2 or said 20 polypeptide is encoded by a nucleotide sequence translatable to at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4 and subsequently cloned by designing at least one oligonucleotide probe based on said nucleotide sequence and using same to clone a nucleotide sequence encoding said LERK or polypeptide from a DNA or RNA library.

- 25 This aspect of the present invention extends to recombinant NLERK2 or a polypeptide having NLERK2-like properties but does not extend to known LERKs such as LERK1, LERK2, LERK3 or LERK4.

- 8 -

In a particularly preferred embodiment, the present invention provides NLERK2 which comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or having at least about 70% similarity, preferably at least about 80% similarity, more preferably at least about 90% similarity or most preferably at least about 95 to 100% similarity to all or part of the 5 sequence set forth in SEQ ID NO:6. This aspect encompasses NLERK2 and derivatives thereof such as fragments, parts, portions, mutants and homologues.

In a related embodiment, the present invention contemplates a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding the amino acid 10 sequence set forth in SEQ ID NO:6 or to an amino acid sequence having at least about 70% or above similarity to all or part of the amino acid sequence set forth in SEQ ID NO:6.

As stated above, the present invention further contemplates a range of derivatives of NLERK2. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the 15 NLERK2 polypeptide and corresponding genetic sequence. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to NLERK2 or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding NLERK2. "Additions" to amino acid sequences or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to 20 "NLERK2" includes reference to all derivatives thereof including functional derivatives or NLERK2 immunologically interactive derivatives.

Analogues of NLERK2 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, 25 polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde 30 followed by reduction with NaBH<sub>4</sub>; amidination with methylacetimidate; acylation with acetic

- 9 -

anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylolation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with NaBH<sub>4</sub>.

5

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

10 The carboxyl group may be modified by carbodiimide activation via O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other 15 substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

20 Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetraniromethane to form 25 a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation 25 with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, 30 ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-

- 10 -

isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 1.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having  $(CH_2)_n$  spacer groups with n=1 to 5 n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of  $C_\alpha$  10 and  $N_\epsilon$ -methylamino acids, introduction of double bonds between  $C_\alpha$  and  $C_\beta$  atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

15 The present invention further contemplates chemical analogues of NLERK2 capable of acting as antagonists or agonists of NLERK2 or which can act as functional analogues of NLERK2. Chemical analogues may not necessarily be derived from NLERK2 but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of NLERK2. Chemical analogues may be 20 chemically synthesised or may be detected following, for example, natural product screening.

The identification of NLERK2 permits the generation of a range of therapeutic molecules capable of modulating expression of NLERK2 or modulating the activity of NLERK2. Modulators contemplated by the present invention includes agonists and antagonists of 25 NLERK2 expression. Antagonists of NLERK2 expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of NLERK2 include molecules which overcome any negative regulatory mechanism. Antagonists of NLERK2 include antibodies and inhibitor peptide fragments.

- 11 -

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane- carboxylate	Cpro	L-N-methyleasparagine	Nmasn
		L-N-methyleaspartic acid	Nmasp
10 aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl- carboxylate	Norb	L-N-methylglutamine	Nmgln
cyclohexylalanine		L-N-methylglutamic acid	Nmglu
cyclopentylalanine	Cpen	Chexa L-N-methylhistidine	Nmhis
		L-N-methylisoleucine	Nmile
15 D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20 D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
25 D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30 D-threonine	Dthr	L-norleucine	Nle

- 12 -

	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgabu
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
5	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpen
	D- $\alpha$ -methylasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methylaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
10	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
15	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
20	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- $\alpha$ -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
	D- $\alpha$ -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
25	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
30	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser

- 13 -

D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
5 D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nieu	D-N-methylthreonine	Dnmthr
10 D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-naphthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
$\gamma$ -aminobutyric acid	Gabu	N-(p-hydroxyphenyl)glycine	Nhtyr
L-t-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
15 L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methylasparagine	Masn
L- $\alpha$ -methylaspartate	Masp	L- $\alpha$ -methyl-t-butylglycine	Mtbug
L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
20 L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L- $\alpha$ -methylleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
25 L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr

- 14 -

L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl)	Nnbhm	N-(N-(3,3-diphenylpropyl)	Nnbhe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmhc		
5 ethylamino)cyclopropane			

These types of modifications may be important to stabilise NLERK2 if administered to an individual or for use as a diagnostic reagent.

10

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

15

Another embodiment of the present invention contemplates a method for modulating expression of NLERK2 in a human, said method comprising contacting the NLERK2 gene encoding NLERK2 with an effective amount of a modulator of NLERK2 expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate 20 expression of NLERK2. For example, a nucleic acid molecule encoding NLERK2 or a derivative thereof may be introduced into a cell to enhance the ability of that cell to survive, conversely, NLERK2 antisense sequences such as oligonucleotides may be introduced to decrease the survival capacity of any cell expressing the endogenous NLERK2 gene.

25 Another aspect of the present invention contemplates a method of modulating activity of NLERK2 in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease NLERK2 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of NLERK2 or its receptor or a chemical analogue or 30 truncation mutant of NLERK2 or its receptor.

- 15 -

Accordingly, the present invention contemplates a pharmaceutical composition comprising NLERK2 or a derivative thereof or a modulator of NLERK2 expression or NLERK2 activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to as the active ingredients.

5

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and 10 must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required 15 particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the 20 compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by 25 incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously 30 sterile-filtered solution thereof.

- 16 -

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 ug and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

- 17 -

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

10

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which 15 bodily health is impaired as herein disclosed in detail.

20

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said 25 ingredients.

30

- 18 -

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating NLERK2 expression or NLERK2 activity. The vector may, for example, be a viral vector.

5

Still another aspect of the present invention is directed to antibodies to NLERK2 and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to NLERK2 or may be specifically raised to NLERK2 or derivatives thereof. In the case of the latter, NLERK2 or its derivatives may first need to be 10 associated with a carrier molecule. The antibodies and/or recombinant NLERK2 or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

For example, NLERK2 and its derivatives can be used to screen for naturally occurring antibodies to NLERK2. These may occur, for example in some autoimmune diseases.

15 Alternatively, specific antibodies can be used to screen for NLERK2. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of NLERK2 levels may be important for diagnosis of certain cancers or a predisposition to cancers or for monitoring certain therapeutic protocols.

20 Antibodies to NLERK2 of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for 25 immunotherapy and may also be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimen.

For example, specific antibodies can be used to screen for NLERK2 proteins. The latter would be important, for example, as a means for screening for levels of NLERK2 in a cell 30 extract or other biological fluid or purifying NLERK2 made by recombinant means from

- 19 -

culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, 5 polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of NLERK2.

10

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of NLERK2, 15 or antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

20 The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

25

Another aspect of the present invention contemplates a method for detecting NLERK2 in a biological sample from a subject said method comprising contacting said biological sample with an antibody specific for NLERK2 or its derivatives or homologues for a time and under conditions sufficient for an antibody-NLERK2 complex to form, and then detecting said 30 complex.

- 20 -

The presence of NLERK2 may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, includes both single-site and two-site or "sandwich" assays of the non-competitive 5 types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, 10 and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a 15 detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known 20 amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain NLERK2 including cell extract, tissue biopsy or possibly serum, saliva, mucosal 25 secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

- 21 -

In the typical forward sandwich assay, a first antibody having specificity for the NLERK2 or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports 5 may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2- 10 40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

15

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. 20 Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its 25 chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, 30 generally by means of glutaraldehyde or periodate. As will be readily recognized, however,

- 22 -

a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

15

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect NLRK2 gene or its derivatives. Alternative methods or methods used in conjunction include direct nucleotide sequencing or mutation scanning such as single stranded

conformation polymorphoms analysis (SSCP) as specific oligonucleotide hybridisation, as methods such as direct protein truncation tests.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

15

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human NLERK2 gene portion, which NLERK2 gene portion is capable of encoding an NLERK2 polypeptide or a functional or immunologically interactive derivative thereof.

20

Preferably, the NLERK2 gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said NLERK2 gene portion in an appropriate cell.

25 In addition, the NLERK2 gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells  
30 comprising same.

- 24 -

The present invention also extends to any or all derivatives of NLERK2 including mutants, part, fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence.

5

The NLERK2 and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents and will be especially useful in the detection of a corresponding ERK. For example, recombinant NLERK2 may be bound or fused to a reporter molecule capable of producing an identifiable signal, contacted with a cell or group 10 of cells putatively carrying ERKs and screening for binding of the labelled LERK to the ERK. Alternatively, labelled NLERK2 may be used to screen expression libraries of putative ERK genes or functional parts thereof.

LERKs are important for the proliferation, differentiation and survival of a diverse array of 15 cell types. Accordingly, it is proposed that NLERK2 or its functional derivatives be used to regulate development, maintenance or regeneration in an array of different cells and tissues *in vitro* and *in vivo*. For example, NLERK2 is contemplated to be useful in modulating neuronal proliferation, differentiation and survival.

20 Soluble NLERK2 polypeptides are also contemplated to be useful in the treatment of disease, injury or abnormality in the nervous system, e.g. in relation to central or peripheral nervous system to treat Cerebral Palsy, trauma induced paralysis, vascular ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, Multiple Sclerosis, peripheral neuropathies associated with diabetes, 25 heavy metal or alcohol toxicity, renal failure and infectious diseases such as herpes, rubella, measles, chicken pox, HIV or HTLV-1.

Membrane bound NLERK2 may be used *in vitro* on nerve cells or tissues to modulate proliferation, differentiation or survival, for example, in grafting procedures or transplantation.

30

- 25 -

As stated above, the NLERK2 of the present invention or its functional derivatives may be provided in a pharmaceutical composition comprising the LERK together with one or more pharmaceutically acceptable carriers and/or diluents. In addition, the present invention contemplates a method of treatment comprising the administration of an effective amount of 5 a LERK of the present invention. The present invention also extends to antagonists and agonists of LERKs and their use in therapeutic compositions and methodologies.

A further aspect of the present invention contemplates the use of NLERK2 or its functional derivatives in the manufacture of a medicament for the treatment of NLERK2 mediated 10 conditions defective or deficient.

The present invention is further described by the following non-limiting Figures and Examples.

15 **In the Figures:**

**Figure 1** is a representation of the amino acid sequence of NLERK2 [SEQ ID NO: 6] of the present invention in comparison to known LERKs. LERK-1/B61 is described in International Patent Publication No. WO 95/06085; MCEK7/ELF1 in WO 96/10911 and NLERK1/ELF2 20 in WO 96/01839. The putative leader sequences, transmembrane sequences and GPI anchor sequences are shaded and boxed. Areas of amino acid sequence conservation are boxed, dashes signify gaps introduced to produce the alignment and potential N-glycosylation sites are underlined.

25 **Figure 2** is a photographic representation showing expression of NLERK2. Panel A represents SDS-PAGE of extracellular medium from COS cells transfected with pEF sol NLERK2 cDNA (tracks a and b) or pEF fl NLERK2 cDNA (tracks c and d). Panel B represent SDS-PAGE of whole cell lysates from COS cells transfected with pEF sol NLERK2 cDNA (tracks a and b) or pEF fl NLERK2 cDNA (tracks c and d). (a, b are from two 30 independent pEF sol NLERK2 clones and c, d are from two independent pEF fl NLERK2

- 26 -

clones). SDS-PAGE gels were transferred to PVDF filters and blotted with M2 anti-FLAG™ antibody (IBI/Kodak, CT, USA) followed by HRP-conjugated rabbit anti-mouse immunoglobulin (Dako code PO260, Denmark) and binding detected by enhanced chemiluminescence as described for Amersham ECL reagent (Amersham, Buckinghamshire, 5 England).

Figure 3 is a schematic representation showing structure of NLERK2 cDNA and location of NLERK2 clones.

- 10 Figure 4 is a representation of the nucleotide sequence [SEQ ID NO:5] and corresponding amino acid sequence [SEQ ID NO:14] of full length NLERK2 cDNA. The leader sequence is lightly underlined, the transmembrane domain is heavily underlined and the potential N-glycosylation site is indicated by a bold N.
- 15 Figure 5 is a representation of the N-terminal amino acid sequence of pEF soINLERK2. The underlined portion is the C-terminal portion of the IL-3 signal sequence. The dotted underlined sequence is the sequence of the FLAG tag used to detect the expressed molecule. The double underlined sequence is the 5' cloning site for the NLERK2 cDNA. The non-underlined residues are the mature 5' coding region.

- 27 -

A summary of SEQ ID Nos used in this specification is given in Table 2.

**TABLE 2**  
**SUMMARY OF SEQ ID NOs**

5

	SEQUENCE	SEQ ID NO:
10	VXWXSXN	1
	DXXDIXCP	2
	EXYXLYXVXXXXXXXC	3
	KFQXXXXXXXXGXEFXXXHXXXXI	4
	Nucleotide sequence of full length NLERK2	5
15	Amino acid sequence of NLERK2	6
	Oligonucleotide	7
	Oligonucleotide	8
	Oligonucleotide	9
	Oligonucleotide	10
20	Oligonucleotide	11
	Oligonucleotide	12
	Oligonucleotide	13
	3' end of NLERK2 gene	14
	5' end of NLERK2 gene	15
25	Signal sequence of murine IL-3	16
	FLAG epitope	17

- 28 -

Single and triple letter abbreviations for amino acid residues are used in the subject specification, as defined in Table 3.

**TABLE 3**  
**AMINO ACID ABBREVIATIONS**

5

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
10	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
15	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
20	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
25	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X

30

- 29 -

#### EXAMPLE 1

#### RATIONALE

An approach was used based on conserved amino acid sequence elements in the LERKs to seek additional members of the family, reasoning that the large number of ERKs (at least 12) implied an equally large number of LERKs. The known LERK sequences were aligned and are shown in Figure 1. It was noted that there were small conserved amino acid sequence blocks, namely

- 10 (i) VXWXSXN [SEQ ID NO:1];  
(ii) DXXDIXCP [SEQ ID NO:2];  
(iii) EXYXLYXVXXXXXXC [SEQ ID NO:3]; and/or  
(iv) KFQXXXXXXXXGXEFXXXHXYXYI [SEQ ID NO:4].

These amino acid sequence blocks were used to individually search the NCBI-Genbank data bank for any nucleotide sequences that, when translated in any of the six possible ways into 15 amino acids (forward in each of three reading frames and backward in each of three reading frames), could encode a peptide corresponding to the above sequence blocks. For this purpose, the Pearson TFASTA search routine (Pearson WR and Lipman DJ *Proc. Natl. Acad. Sci. USA* 85: 2444-2448, 1988; and Pearson WR *Methods in Enzymology* 183: 63-98, 1990) was used. Using SEQ ID NO:4 resulted in highest scores for human LERK2 (Gene Bank 20 Code No. HSU09304), rat ELK-L (Gene Bank Code No. RSU07560), human ELK-L (Gene Bank Code No. HUMEFL3), human EHK1-L (Gene Bank Code No. HUMEFL2), mouse LERK2 (Gene Bank Code No. MLERK2), human B61 (Gene Bank Code No. HUMB61), mouse ERF-1 (Gene Bank Code No. MMU14941), mouse cek 5-L (Gene Bank Code No. MMU12983).

25

The next highest score was for deposited Gene Bank Code No. 269886NCBI which represents a human-expressed sequence tag (EST) derived from a 73 day post-natal female whole brain cDNA library. Alignment and translation of an amino acid sequence predicted to be encoded by the EST (Gene Bank Code No. 269886NCBI) demonstrates it to be related to the known 30 LERKs (Figure 1). The sequence does diverge, however, and hence encodes a novel human

- 30 -

LERK, referred to herein as "NLERK2" (Figures 1 and 4).

#### EXAMPLE 2

##### CLONING FULL LENGTH NLERK2 cDNA

5 Pooled oligos were used to probe library 53 which is a human fetal brain cDNA library. The oligos were synthesized according to the cDNA sequence of the expressed sequence tag [EST tag no. 269886NCBI]. The oligonucleotide probes comprise the following sequences:

- |         |    |                                |               |
|---------|----|--------------------------------|---------------|
| (i)     | 5' | GGCAGGTTCTCCTCCCCAGGGCTCCCA 3' | [SEQ ID NO:7] |
| 10 (ii) | 5' | GTAATCGTGGTGCGAGCG 3'          | [SEQ ID NO:8] |
| (iii)   | 5' | GGCATGAAGGTGCTTCTCCGA 3'       | [SEQ ID NO:9] |

A number of duplicate positive hybridizing plaques were picked and purified on CsCl gradient and sequenced on both strands. Overlapping cDNA sequences from 5 independent clones 15 were obtained encompassing the entire coding region and were sequenced on both strands (see Figure 4).

#### EXAMPLE 3

##### GENERATION OF EXPRESSION CONSTRUCTS

20 Constructs were generated to express full length and soluble forms of the protein. A derivative of the mammalian expression vector pEF-BOS (Mizushima *et al*, *Nucl. Acids. Res.* 18: 5322, 1990) was engineered to contain DNA encoding the signal sequence of murine IL-3 (MVLASSTTSIHTMLLLLMLFHLGLQASIS [SEQ ID NO:16]) and the FLAG epitope (DYKDDDDK [SEQ ID NO:17]) followed by a unique Xba 1 cloning site. This vector was 25 named pEF/IL3SIG/FLAG.

- 31 -

PCR was performed using oligos to amplify DNA fragments encoding the entire mature coding region from L28 to V340 using clones 53.1 and 53.8. The oligonucleotide probes comprise the following sequences:

- 5 (i) 5' AGCTTCTAGACTCAACCTGGAGCCTGTCTAC 3'  
[SEQ ID NO:10]  
(ii) 5' AGCTTCTAGATCATACCTTGATGATGTTGG 3'  
[SEQ ID NO:11]

10 PCR was used to amplify the extracellular domain without the transmembrane or cytoplasmic region. The sequences of the oligonucleotides used in this PCR are as follows:

- (i) 5' AGCTTCTAGACTCAACCTGGAGCCTGTCTAC 3'  
[SEQ ID NO:12]  
15 (ii) 5' AGCTTCTAGATCAGCTGGAGGGGGCAGGGGGCC 3'  
[SEQ ID NO:13]

Full length and soluble forms were digested with XbaI. Both products were cloned in frame into the XbaI site of pEF/IL3SIG/FLAG to yield pEF fl NLERK2 and pEF sol NLERK2. The  
20 vectors are expressed in COS cells as described below.

In order to confirm that full-length and soluble forms of NLERK2 could be produced using the expression vectors pEF-flNLERK-2 and pEF-solNLERK-2, COS cells were transiently transfected with these constructs. Briefly, COS cells from a confluent 175 cm<sup>2</sup> tissue culture  
25 flask were resuspended in PBS and electroporated (BioRad Gene pulser; 500 µF, 300 V) with 20 µg of uncut expression vector in a 0.4 cm cuvette (BioRad). After 2 to 3 days at 37°C in a fully humidified incubator containing 10% v/v CO<sub>2</sub> in air, cells were used for analyses of protein expression. Conditioned medium was collected by centrifugation, concentrated ten-fold, and stored sterile at 4°C. Cells were also harvested and lysed for 5 min in 500 µl of 50  
30 mM Tris HCl pH7.4 containing 150 mM NaCl, 2 mM EDTA and 1% v/v Triton X-100. The

- 32 -

intact nuclei were removed by centrifugation at 10,000g for 5 min. The cell lysate and conditioned medium were electrophoresed on pre-cast 12% w/v polyacrylamide gels (BioRad). The resolved proteins were then electroblotted onto PVDF membranes, which were then blocked with 5% skim milk, 0.1% v/v Tween 20 in PBS, rinsed and incubated with 5 µl of 5 anti-FLAG M2 antibody (IBI) in 2.5 ml of PBS containing 5% skim milk and 0.1% v/v Tween 20. The membrane was then rinsed and incubated with peroxidase-conjugated human anti-mouse Ig in 5% skim milk, 0.1% v/v Tween in PBS, rinsed and incubated with ECL reagent (Amersham, Buckinghamshire, UK) or 1 min. Filters were then blotted dry and exposed to autoradiographic film for 1 min.

10

Figure 2 shows expression of NLERK2 in COS cells. The apparent molecular mass of soluble and full length NLERK2 were 32 and 50 kDa, respectively, suggesting glycosylation.

Figure 5 shows the N-terminal amino acid sequence of anti-FLAG M2 antibody purified 15 soluble NLERK2 from pEF solNLERK2. This protein is transiently expressed in COS cells (Figure 2). Single underlined sequence is the C-terminal portion of the IL-3 signal sequence which was cleaved before the Alanine. The dotted underlined sequence is sequence of the FLAG tag used to detect the expressed molecule. The double underlined sequence is the 5' cloning site for the NLERK2 cDNA. The non-underlined residues are the mature 5' coding 20 region (lacking the leader sequence). The italicised Asparagine (*N*) was modified for cloning reasons from the Serine residue of the sequence given in Figure 4.

The full length nucleotide sequence [SEQ ID NO:5] and corresponding amino acid sequence [SEQ ID NO:6] is shown in Figure 4.

25

- 33 -

**EXAMPLE 4**  
**FLANKING REGIONS OF NLERK2 GENE**

The 5' and 3' portions of the NLERK2 genomic gene were determined and are shown in SEQ ID NO:15 and SEQ ID NO:14, respectively.

5

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this 10 specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

- 34 -

## SEQUENCE LISTING

### (1) GENERAL INFORMATION:

(i) APPLICANT (countries other than US): AMRAD OPERATIONS PTY. LTD.  
(US only): NICOS ANTHONY NICOLA

(ii) TITLE OF INVENTION: NOVEL RECEPTOR LIGANDS AND GENETIC  
SEQUENCES ENCODING SAME

(iii) NUMBER OF SEQUENCES: 17

### (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE  
(B) STREET: 1 LITTLE COLLINS STREET  
(C) CITY: MELBOURNE  
(D) STATE: VICTORIA  
(E) COUNTRY: AUSTRALIA  
(F) ZIP: 3000

### (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

### (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT INTERNATIONAL  
(B) FILING DATE: 19-JUL-1996

### (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: PN4263  
(B) FILING DATE: 20-JUL-1995  
(A) APPLICATION NUMBER: PN6847  
(B) FILING DATE: 27-NOV-1995

- 35 -

(A) APPLICATION NUMBER: PN7299  
(B) FILING DATE: 22-DEC-1995  
(A) APPLICATION NUMBER: PN7890  
(B) FILING DATE: 05-FEB-1996

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES DR, E JOHN L  
(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: +61 3 9254 2777  
(B) TELEFAX: +61 3 9254 2770

- 36 -

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 7 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Val Xaa Trp Xaa Ser Xaa Asn  
1 5

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 8 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Xaa Xaa Asp Ile Xaa Cys Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 16 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Xaa Tyr Xaa Leu Tyr Xaa Val Xaa Xaa Xaa Xaa Xaa Xaa Cys  
1 5 10 15

- 37 -

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Lys	Phe	Gln	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Gly	Xaa	Glu	Phe	Xaa	Xaa
1														15
Xaa His Xaa Tyr Tyr Xaa Xaa Ile														
20														

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1021 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATG	GGC	CCC	CCC	CAT	TCT	GGG	CCG	GGG	GGC	GTG	CGA	GTC	GGG	GCC	CTC	48
Met	Gly	Pro	Pro	His	Ser	Gly	Pro	Gly	Gly	Val	Arg	Val	Gly	Ala	Leu	
1															15	
CTC	CTA	CTG	GGG	GTT	TTG	GGG	CTG	GTG	TCT	GGG	CTC	AGC	CTG	GAG	CCT	96
Leu	Leu	Leu	Gly	Val	Leu	Gly	Leu	Val	Ser	Gly	Leu	Ser	Leu	Glu	Pro	
20															30	
GTC	TAC	TGG	AAC	TCG	GCG	AAT	AAG	AGG	TTC	CAG	GCA	GAG	GGT	GGT	TAT	144
Val	Tyr	Trp	Asn	Ser	Ala	Asn	Lys	Arg	Phe	Gln	Ala	Glu	Gly	Gly	Tyr	
35															45	

- 38 -

GTG CTG TAC CCT CAG ATC GGG GAC CGG CTA GAC CTA CTC TGC CCC CGG		192
Val Leu Tyr Pro Gln Ile Gly Asp Arg Leu Asp Leu Leu Cys Pro Arg		
50	55	60
GCC CGG CCT CCT GGC CCT CAC TCC TCT CCT AAT TAT GAG TTC TAC AAG		240
Ala Arg Pro Pro Gly Pro His Ser Ser Pro Asn Tyr Glu Phe Tyr Lys		
65	70	75
CTG TAC CTG GTA GGG GGT GCT CAG GGC CGA CGC TGT GAG GCA CCC CCT		288
Leu Tyr Leu Val Gly Gly Ala Gln Gly Arg Arg Cys Glu Ala Pro Pro		
85	90	95
GCC CCA AAC CTC CTT CTC ACT TGT GAT CGC CCA GAC CTG GAT CTC CGC		336
Ala Pro Asn Leu Leu Leu Thr Cys Asp Arg Pro Asp Leu Asp Leu Arg		
100	105	110
TTC ACC ATC AAG TTC CAG GAG TAT AGC CCT AAT CTC TGG GGC CAC GAG		384
Phe Thr Ile Lys Phe Gln Glu Tyr Ser Pro Asn Leu Trp Gly His Glu		
115	120	125
TTC CGC TCG CAC CAC GAT TAC TAC ATC ATT GCC ACA TCG GAT GGG ACC		432
Phe Arg Ser His His Asp Tyr Tyr Ile Ile Ala Thr Ser Asp Gly Thr		
130	135	140
CGG GAG GGC CTG GAG AGC CTA CAG GGA GGT GTG TGC CTA ACC AGA GGC		480
Arg Glu Gly Leu Glu Ser Leu Gln Gly Gly Val Cys Leu Thr Arg Gly		
145	150	155
ATG AAG GTG CTT CTC CGA GTG GGA CAA AGT CCC CGA GGA GGG GCT GTC		528
Met Lys Val Leu Leu Arg Val Gly Gln Ser Pro Arg Gly Gly Ala Val		
165	170	175
CCC CGA AAA CCT GTG TCT GAA ATG CCC ATG GAA AGA GAC CGA GGG GCA		576
Pro Arg Lys Pro Val Ser Glu Met Pro Met Glu Arg Asp Arg Gly Ala		
180	185	190
GCC CAC AGC CTG GAG CCT GGG AAG GAG AAC CTG CCA GGT GAC CCC ACC		624
Ala His Ser Leu Glu Pro Gly Lys Glu Asn Leu Pro Gly Asp Pro Thr		
195	200	205
AGC AAT GCA ACC TCC CGG GGT GCT GAA GGC CCC CTG CCC CCT CCC AGC		672
Ser Asn Ala Thr Ser Arg Gly Ala Glu Gly Pro Leu Pro Pro Pro Ser		
210	215	220

- 39 -

ATG CCT GCA GTG GCT GGG GCA GCA GGG GGG CTG GCG CTG CTC TTG CTG		720
Met Pro Ala Val Ala Gly Ala Ala Gly Leu Ala Leu Leu Leu Leu		
225 230 235 240		
GGC GTG GCA GGG GCT GGG GGT GCC ATG TGT TGG CGG AGA CGG CGG GCC		768
Gly Val Ala Gly Ala Gly Gly Ala Met Cys Trp Arg Arg Arg Arg Ala		
245 250 255		
AAG CCT TCG GAG AGT CGC CAC CCT GGT CCT GGC TCC TTC GGG AGG GGA		816
Lys Pro Ser Glu Ser Arg His Pro Gly Pro Gly Ser Phe Gly Arg Gly		
260 265 270		
GGG TCT CTG GGC CTG GGG GGT GGA GGT GGG ATG GGA CCT CGG GAG GCT		864
Gly Ser Leu Gly Leu Gly Gly Gly Gly Met Gly Pro Arg Glu Ala	,	
275 280 285		
GAG CCT GGG GAG CTA GGG ATA GCT CTG CGG GGT GGC GGG GCT GCA GAT		912
Glu Pro Gly Glu Leu Gly Ile Ala Leu Arg Gly Gly Ala Ala Asp		
290 295 300		
CCC CCC TTC TGC CCC CAC TAT GAG AAG GTG AGT GGT GAC TAT GGG CAT		960
Pro Pro Phe Cys Pro His Tyr Glu Lys Val Ser Gly Asp Tyr Gly His		
305 310 315 320		
CCT GTG TAT ATC GTG CAG GAT GGG CCC CCC CAG AGC CCT CCA AAC ATC		1008
Pro Val Tyr Ile Val Gln Asp Gly Pro Pro Gln Ser Pro Pro Asn Ile		
325 330 335		
TAC TAC AAG GTA TGA		
Tyr Tyr Lys Val *		
340		

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 340 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Gly Pro Pro His Ser Gly Pro Gly Gly Val Arg Val Gly Ala Leu  
                   1                     5                         10                         15

- 40 -

Leu	Leu	Leu	Gly	Val	Leu	Gly	Leu	Val	Ser	Gly	Leu	Ser	Leu	Glu	Pro
				20			25				30				
Val	Tyr	Trp	Asn	Ser	Ala	Asn	Lys	Arg	Phe	Gln	Ala	Glu	Gly	Gly	Tyr
			35			40					45				
Val	Leu	Tyr	Pro	Gln	Ile	Gly	Asp	Arg	Leu	Asp	Leu	Cys	Pro	Arg	
			50			55				60					
Ala	Arg	Pro	Pro	Gly	Pro	His	Ser	Ser	Pro	Asn	Tyr	Glu	Phe	Tyr	Lys
	65				70				75			80			
Leu	Tyr	Leu	Val	Gly	Gly	Ala	Gln	Gly	Arg	Arg	Cys	Glu	Ala	Pro	Pro
			85			90				95					
Ala	Pro	Asn	Leu	Leu	Leu	Thr	Cys	Asp	Arg	Pro	Asp	Leu	Asp	Leu	Arg
			100			105				110					
Phe	Thr	Ile	Lys	Phe	Gln	Glu	Tyr	Ser	Pro	Asn	Leu	Trp	Gly	His	Glu
		115			120				125						
Phe	Arg	Ser	His	His	Asp	Tyr	Tyr	Ile	Ile	Ala	Thr	Ser	Asp	Gly	Thr
	130				135				140						
Arg	Glu	Gly	Leu	Glu	Ser	Leu	Gln	Gly	Val	Cys	Leu	Thr	Arg	Gly	
	145			150			155				160				
Met	Lys	Val	Leu	Leu	Arg	Val	Gly	Gln	Ser	Pro	Arg	Gly	Ala	Val	
			165			170				175					
Pro	Arg	Lys	Pro	Val	Ser	Glu	Met	Pro	Met	Glu	Arg	Asp	Arg	Gly	Ala
			180			185				190					
Ala	His	Ser	Leu	Glu	Pro	Gly	Lys	Glu	Asn	Leu	Pro	Gly	Asp	Pro	Thr
			195			200				205					
Ser	Asn	Ala	Thr	Ser	Arg	Gly	Ala	Glu	Gly	Pro	Leu	Pro	Pro	Pro	Ser
	210			215			220								
Met	Pro	Ala	Val	Ala	Gly	Ala	Ala	Gly	Gly	Leu	Ala	Leu	Leu	Leu	
	225			230			235				240				
Gly	Val	Ala	Gly	Ala	Gly	Ala	Met	Cys	Trp	Arg	Arg	Arg	Arg	Ala	
			245			250				255					

- 41 -

Lys	Pro	Ser	Glu	Ser	Arg	His	Pro	Gly	Pro	Gly	Ser	Phe	Gly	Arg	Gly
260															
Gly	Ser	Leu	Gly	Leu	Gly	Gly	Gly	Gly	Met	Gly	Pro	Arg	Glu	Ala	
275															
Glu	Pro	Gly	Glu	Leu	Gly	Ile	Ala	Leu	Arg	Gly	Gly	Ala	Ala	Asp	
290															
Pro	Pro	Phe	Cys	Pro	His	Tyr	Glu	Lys	Val	Ser	Gly	Asp	Tyr	Gly	His
305															
Pro	Val	Tyr	Ile	Val	Gln	Asp	Gly	Pro	Pro	Gln	Ser	Pro	Pro	Asn	Ile
	325														
Tyr	Tyr	Lys	Val	*											
		340													

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleotide
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCAGGTTCT CCTTCCCCAG GCTCCCA

27

## (2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleotide
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTAGTAATCG TGGTGCGAGC G

21

- 42 -

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGCATGAAGG TGCTTCTCCG A

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCTTCTAGA CTCAACCTGG AGCCTGTCTA C

31

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGCTTCTAGA TCATACCTTG TAGTAGATGT TTGG

34

- 43 -

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 31 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AGCTTCTAGA CTCAACCTGG AGCCTGTCTA C

31

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AGCTTCTAGA TCAGCTGGGA GGGGGCAGGG GGCC

34

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 1692 base pairs  
(B) TYPE: nucleotide  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGAGGGCTCC TCTCACGTGG CTATCCTGAA TCCAGCCCTT

40

CCTGGGGTGC TCCTCCAGTT TAATTCTGG TTTGAGGGAC ACCTCTAACCA TCTCGCCCC

100

- 44 -

CTGTGCCCCC CCAGCCCC TT CACTCCTCCC GGGTGCTGTC CTCGTCTCCA CTTTTAGGAT	160
TCCTTAGGAT TCCCCACTGCC CCACCTCCTG CCCTCCCGTT TGGCCATGGG TGCCCCCCTC	220
TGTCTCAGTG TCCCTGGATC CTTTTTCCCTT GGGGAGGGGC ACAGGCTCAG CCTCCTCTCT	280
GACCATGAMC CAGGGATCCT TGTCCCCCTC AMCCACCCAG AGCTAGGGGG CGGGAACAGC	340
CCACCTTTG GTTGGCACCG CCTTCTTTCT GCCTCTCACT GGTTTTCTCT TCTCTATCTC	400
TTATTCTTTC CCTCTCTTCC GTCTCTAGGT CTGTTCTTCT TCCCTAGCAT CCTCCTCCCC	460
ACATCTCCTT TCACCCCTTT GGCTTCTTAT CCTGTGCCTC TCCCATCTCC TGGGTGGGS	520
SATCAAAGCA TTTCTCCCT TAGCTTCAS CCCCCCTCT GACCTCTCAT ACCAACCACT	580
CCCCTCAGTC TSYCAAAAAT GGGGGCCTTA TGGGGAAAGGC TCTGACACTC CACCCAGCT	640
CAGGCCATGG GCAGCAGGGC TCCATTCTCT GGCTGGCCC AGGCCTCTAC ATACTTACTC	700
CAGCCATTG GGGTGGTTGG GTCATGACAG CTACCATGAG AAGAAGTGTGTC CCGTTTTGTC	760
CAGTGGCAA TAGCAAGATA TGAACCGGTC GGGACATGTA TGGACTTGGT CTGATGCTGA	820
ATGGGCCACT TGGGACCGGA AGTGACTTGC TCCAGACAAG AGGTGACCAAG GCCCAGACAG	880
AAATGGCCTG GGAAGTAGCA GAAGCAGTGC AGCAGGAACG GGAAGTGCCCT TTCATCCAGG	940
ACAGGAAGTA GCACCTCTGA WRCNAGGAAG TGRCTGTCT RGAACACCAA GTGGCWTART	1000
CTGGGGGATC AGGAGGTGGG AGGTGGATGG TTCTTATTCT GTGGAGAAGA AGGGCGGGAA	1060
GAACCTCCTT TCAGGAGGAA GCTGGAACCT ACTGACTGTA AGAGGTTAGA GGTGGACCGA	1120
GAAGGACTTT TCCCAGTCTT CAGTGGCACT TCCCAAGATC TCCCTCCCT TGTGCTCTGT	1180
GCTGATTTA GGACAGCTAA GATGACTGCC ATGTGCTGTG GCAGGCCTAA TTTGTCTTGT	1240
TCTTCCCTT CCATATCCCA GTATAATCTC TGTTAATCAA CAGGACTACC CCAAGAACCC	1300
ATGTGCTCTC CCGAGTAACC CAGATGGCTG TCTTGTTCAT TCCATCCTAC ATTTCTGACT	1360
CCTTTCAGAC TCAACACAGT TCCCTCTTA GTGACCACAA TGGTGGCCTA CTGCCTGGTC	1420
TAGCTGACAG TGGTACTTAG CAMAGGCCAC TGTTCCATA GTGACCAGCT GATACCTCTT	1480

- 45 -

CCTGCCCTCT AGTGTGCAAT TGGGTGTTGC CTCAGTTCC TCCCAGCTCA GTTTTATTAG	1540
ATCAAACCTG TTGTTGGGCA CCAGGTTGTC CACCTCAATC ACCAGCCAAG ATGGTTGCTT	1600
TGTCCACCAG AGGTCAAGTT CACCTCTCTG GTGCTGTAGT TCCCAGCTCC TTCCTGATTT	1660
TTCTAATGGA ATTGATATC AAGCTTATCG AT	1692

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 399 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GTGGAGCCA CGCGGTGGCG GTCGCTCTAG AATAGTGGAT CCCCCGGGCT GCAGGAATT	60
CAGCCCCCTTC CTCGCTCCCT GGTCCGGCGC CCCATGCCGC CCCCCGGGGG TCCCCGGYTC	120
CCCCAGTCCC CCACTTAGGC GGGATCACAG ATCCCGGGGT GCTGGCGCGT GGGCCGGGGG	180
CGCGTAGGGC GCCTGCAGAC GGCCCCCTGGA AGGGCTCTGG TGGGGCTGAG CGCTCTGCCG	240
CGGGGGCGCG GGCACAGCAG GAAGCAGGTC CGCGTGGGCG CTGGGGGCAT CAGCTACCGG	300
GGTGGTCCGG GCTGAAGAGC CAGGCASCAA GGCAGCCACC CCGGGGGGTG GGCGACTTTG	360
GGGGAGTTGG TGCGCCCCCC CCCAGACCTT GACGGGGTC	399

- 46 -

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Val Leu Ala Ser Ser Thr Thr Ser Ile His Thr Met Leu Leu Leu  
5 10 15

Leu Leu Met Leu Phe His Leu Gly Leu Gln Ala Ser Ile Ser  
20 25

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Tyr Lys Asp Asp Asp Asp Lys  
5

## CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a LERK or part thereof, said nucleotide sequence or its complementary form capable of encoding at least one of the following amino acid sequences:
  - (i) VXWXSXN [SEQ ID NO:1];
  - (ii) DXXDIXCP [SEQ ID NO:2];
  - (iii) EXYXLYXVXXXXXXXXC [SEQ ID NO:3]; and/or
  - (iv) KFQXXXXXXXXGXEFXXXHXYXI [SEQ ID NO:4].wherein X is any amino acid residue.
2. An isolated nucleic acid molecule according to claim 1 encoding at least two of SEQ ID NO:1 to SEQ ID NO:4.
3. An isolated nucleic acid molecule according to claim 1 encoding at least three of SEQ ID NO:1 to SEQ ID NO:4.
4. An isolated nucleic acid molecule according to claim 1 selected from the group consisting of:
  - (i) a nucleic acid molecule having a nucleotide sequence substantially as set forth in SEQ ID NO:5;
  - (ii) a nucleic acid molecule having a nucleotide sequence having at least 75% similarity to the sequence set forth in SEQ ID NO:5; and
  - (iii) a nucleic acid molecule capable of hybridising under low stringency conditions to the nucleotide sequence or its complementary form set forth in SEQ ID NO:5.
5. An isolated nucleic acid molecule according to claim 4 further comprising a sequence of nucleotides as set forth in SEQ ID NO:14 and/or SEQ ID NO:15 or having at least 75%

- 48 -

similarity thereto and/or is capable of hybridising thereto under low stringency conditions.

6. An isolated nucleic acid molecule according to claim 4 or 5 obtainable from the human genome.

7. An isolated nucleic acid molecule according to claim 4 or 5 wherein the human genome is in brain, liver, kidney, neonatal, embryonic, cancer or tumour-derived tissue.

8. A method for cloning a nucleotide sequence encoding a novel LERK said method comprising searching a nucleotide database for a sequence which encodes at least one of amino acid sequences SEQ ID NO:1 to SEQ ID NO:4, designing one or more oligonucleotide primers based on a nucleotide sequence located in the search, screening a nucleic acid library with said one or more oligonucleotides and obtaining a clone therefrom which encodes said novel LERK.

9. A method according to claim 8 wherein the nucleic acid library is a cDNA expression library.

10. A method according to claim 9 wherein the cDNA expression library is of human origin derived from brain, liver, kidney, neonatal, embryonic, tumour or cancer tissue.

11. A method according to claim 8 wherein the oligonucleotide primers are selected from SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 and SEQ ID NO:13.

12. A method according to claim 8 wherein the cloned nucleotide sequence is selected from the listing consisting of:

- (i) a nucleotide sequence substantially as set forth in SEQ ID NO:5;
- (ii) a nucleotide sequence having at least 75% similarity to the sequence set forth in SEQ ID NO:5; and

- 49 -

- (iii) a nucleotide sequence capable of hybridising under low stringency conditions to the nucleotide sequence or its complementary form set forth in SEQ ID NO:5.

13. A recombinant polypeptide having the ability to interact with an ERK wherein said recombinant polypeptide comprises at least one of amino acid sequence SEQ ID NO:1 to SEQ ID NO:4.

14. A recombinant polypeptide according to claim 13 having an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof.

15. A pharmaceutical composition comprising a recombinant polypeptide as defined in claim 12 and one or more pharmaceutically acceptable carriers and/or diluents.

16. A pharmaceutical composition according to claim 15 wherein the polypeptide comprises an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof.

17. A use of a recombinant polypeptide having an amino acid sequence substantially as set forth in SEQ ID NO:6 or a functional part thereof in the manufacture of a medicament for the treatment of disease conditions resulting from NLERK2-deficiency or NLERK2-defectiveness.

18. A nucleic acid comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:14 or having at least 75% similarity thereto or is capable of hybridising thereto under low stringency conditions.

19. A nucleic acid comprising a sequence of nucleotides substantially as set forth in SEQ ID NO:15 or having at least 75% similarity thereto or is capable of hybridising thereto under low stringency conditions.

1/17

2/17	3/17	4/17	5/17
6/17	7/17	8/17	9/17

*Fig. 1*

SUBSTITUTE SHEET (Rule 26)

2/17

LERK-1/B61  
 LERK-3  
 MCEK7L/ELF1  
 AL-1/RAGS  
 LERK-4  
 LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

M	A	A	A	P	L	L	L	L
M	A	P	A	Q	R	P	L	P
M	P	H	V	E	M	L	L	A
							M	R
M	A	R	P	G	Q	R	W	L
							M	A
M	A	V	R	R	D	S	V	
M	G	P	P	H	S	C	G	P

LERK-1/B61  
 LERK-3  
 MCEK7L/ELF1  
 AL-1/RAGS  
 LERK-4  
 LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

—	T	I	H	V	Q	L	N	D	Y	V	D
—	T	V	Q	V	N	V	N	D	Y	L	D
Y	T	V	E	V	S	I	N	D	Y	L	D
—	H	I	D	V	C	I	N	D	Y	L	D
—	V	V	E	L	G	L	N	D	Y	L	D
L	V	I	Y	P	K	I	G	D	K	L	D
L	V	L	Y	P	Q	I	G	D	K	L	D
Y	V	L	Y	P	Q	I	G	D	R	L	D

LERK-1/B61  
 LERK-3  
 MCEK7L/ELF1  
 AL-1/RAGS  
 LERK-4  
 LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

W	Q	C	N	R	P	S	A	K	H	G	P
W	E	C	N	R	P	H	A	P	H	S	P
W	E	C	N	R	P	A	A	P	G	G	P
W	E	C	N	R	P	H	S	P	N	G	P
W	V	C	S	L	P	F	G	H	—	—	—
V	T	C	N	R	P	E	Q	E	—	—	—
L	N	C	A	K	P	D	Q	D	—	—	—
L	T	C	D	R	P	D	L	D	—	—	—

Fig. 1

3/17

M	E	F	L	W	A	P	I	L	G	C	C	S	L	A	A
L	L	V	P	V	P	L	I	P	L	A	Q	G	P	G	G
L	L	L	L	L	I	P	L	R	A	R	N	E	D	P	A
V	A	A	L	W	V	C	V	R	G	Q	—	E	P	G	R
L	L	R	T	V	L	W	A	A	F	L	G	S	P	L	R
K	W	L	V	A	M	V	V	W	A	L	C	R	L	A	T
W	K	Y	C	W	G	V	L	M	V	L	C	R	T	A	I
G	G	V	R	V	G	A	L	L	G	V	L	G	L	V	S
															G
I	I	C	P	H	Y	E	D	H	S	V	A	D	A	A	M
I	Y	C	P	H	Y	N	S	S	G	V	G	P	G	A	G
I	Y	C	P	H	Y	G	A	P	L	P	P	A	E	R	M
V	F	C	P	H	Y	E	D	S	V	P	E	D	K	T	—
I	V	C	P	H	Y	E	G	P	G	P	P	E	G	P	—
I	I	C	P	R	A	E	A	G	R	P	Y	—	—	—	—
I	I	C	P	K	V	D	S	K	T	V	G	Q	Y	—	—
L	L	C	P	R	A	R	P	P	G	P	H	S	S	P	N

E	K	L	S	E	K	F	Q	R	F	T	P	F	T	L	G	K	E	F	K
I	K	F	S	E	K	F	Q	R	Y	S	A	F	S	L	G	Y	E	F	H
L	K	F	S	E	K	F	Q	L	F	T	P	F	S	L	G	F	E	F	R
L	K	F	S	E	K	F	Q	L	F	T	P	F	S	L	G	F	E	F	R
V	Q	F	S	E	K	I	Q	R	F	T	P	F	S	L	G	F	E	F	L
I	R	F	T	I	K	F	Q	E	F	S	P	N	Y	M	G	L	E	F	K
I	K	F	T	I	K	F	Q	E	F	S	P	N	L	W	G	L	E	F	Q
L	R	F	T	I	K	F	Q	E	Y	S	P	N	L	W	G	H	E	F	R

Fig. 1

4/17

D	R	H	T	V	F	W	N	S	S	N	P	K	F	R	N	E	D	Y	-
N	R	H	A	V	Y	W	<u>N</u>	<u>S</u>	<u>S</u>	N	Q	H	L	R	R	E	G	Y	-
D	R	Y	A	V	Y	W	<u>N</u>	<u>R</u>	<u>S</u>	N	P	R	F	Q	V	S	A	V	G
D	R	Y	A	V	Y	W	<u>N</u>	<u>S</u>	<u>T</u>	N	P	R	F	Q	Q	G	D	Y	-
L	R	H	V	V	Y	W	<u>N</u>	<u>S</u>	<u>S</u>	N	P	R	L	L	R	G	D	A	-
N	L	E	P	V	S	W	<u>S</u>	<u>S</u>	L	N	P	K	F	L	S	G	K	G	-
V	L	E	P	I	Y	W	<u>N</u>	<u>S</u>	<u>S</u>	N	S	K	F	L	P	G	Q	G	-
S	L	E	P	V	Y	W	<u>N</u>	<u>S</u>	<u>A</u>	N	K	R	F	Q	A	E	G	G	-
--	--	E	Q	Y	I	L	Y	L	V	E	H	E	E	Y	Q	L	C	Q	
G	G	A	E	Q	Y	V	L	Y	M	V	S	R	N	G	Y	R	T	C	
--	--	--	E	R	Y	I	L	Y	M	V	N	G	E	G	H	A	S	C	D
--	--	--	E	R	Y	V	L	Y	M	V	N	F	D	G	Y	S	S	C	D
--	--	--	E	T	F	A	L	Y	M	V	D	W	P	G	Y	E	S	C	Q
--	--	--	E	Y	Y	K	L	Y	L	V	R	P	E	Q	A	A	A	C	S
--	--	--	E	Y	Y	K	V	Y	M	V	D	K	D	Q	A	D	R	C	T
--	--	--	E	F	Y	K	L	Y	L	V	G	G	A	Q	G	R	R	C	E
E	G	H	S	Y	Y	Y	I	S	K	P	I	H	Q	H	E	D	R	-	-
A	G	H	E	Y	Y	Y	I	S	T	P	T	H	N	L	H	W	K	-	-
P	G	H	E	Y	Y	Y	I	S	A	T	P	P	N	L	V	D	R	P	-
P	G	R	E	Y	F	Y	I	S	S	A	I	P	D	N	G	R	R	S	-
P	G	E	T	Y	Y	Y	I	S	V	P	T	P	E	S	S	G	Q	-	-
K	H	H	D	Y	Y	I	T	S	T	S	<u>N</u>	<u>G</u>	<u>S</u>	<u>L</u>	<u>E</u>	<u>G</u>	<u>L</u>	<u>E</u>	N
K	N	K	D	Y	Y	I	I	S	T	S	<u>N</u>	<u>G</u>	<u>S</u>	<u>L</u>	<u>E</u>	<u>G</u>	<u>L</u>	<u>D</u>	N
S	H	H	D	Y	Y	I	I	A	T	S	D	G	T	R	E	G	L	E	

Fig. 1

5/17

D G G G

P	Q	S	_	K	D	Q	V	R
<u>N</u>	<u>A</u>	<u>S</u>	_	Q	G	F	K	R
H	R	Q	_	R	G	F	K	R
H	I	S	_	K	G	P	K	R
A	E	G	P	R	A	Y	K	R
T	V	L	D	P	N	V	L	_
I	K	K	E	N	T	P	L	_
A	P	P	A	P	N	L	L	_

R	E	G	G	V	C	R	T
Q	E	G	G	V	C	Q	T
L	Q	G	G	V	C	L	T

*Fig. 1*

**SUBSTITUTE SHEET (Rule 26)**

6/17

LERK-1/B61  
 LERK-3  
 MCEK7L/ELF1  
 AL-1/RAGS  
 LERK-4  
 LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

R	-	L	K	V	T	V	-	S	G	K	I
R	-	M	K	V	F	V	C	C	A	S	T
R	-	L	K	V	Y	V	R	P	T	<u>N</u>	E
K	-	L	K	V	F	V	R	P	A	N	S
R	-	L	Q	V	S	V	C	C	K	E	R
R	T	M	K	I	I	M	K	V	G	Q	D
R	A	M	K	I	L	M	K	V	G	Q	D
R	G	M	K	V	L	L	R	V	G	Q	S

LERK-1/B61  
 LERK-3  
 AL-1/RAGS  
 LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

L	L	L	O	T	P	*
R	E	H	L	P	I	A
I	R	L	L	A	T	L
K	H	E	T	V	N	Q
H	S	G	N	N	I	L
P	L	P	P	P	S	M

LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

-	-	-	-	-	R	H	R	K	H	T
-	-	-	-	-	R	H	R	N	H	S
A	K	P	S	E	S	R	H	P	G	P

LERK-2/ELK-L  
 NLERK1/ELF2  
 NLERK2

C	P	H	Y	E	K	V	S	G	D	Y	G
C	P	H	Y	E	K	V	S	G	D	Y	G
C	P	H	Y	E	K	V	S	G	D	Y	G

Fig. 1

7/17

T	H	S	P	Q	A	H	V	N	P	Q	E	K	R	L	A	A	D	D	P
S	H	S	G	E	K	P	V	P	T	L	P	Q	F	T	M	G	P	N	V
T	L	Y	E	A	P	E	P	I	F	T	S	<u>N</u>	<u>S</u>	<u>S</u>	C	S	G	L	G
C	M	K	T	I	G	V	H	D	R	V	F	D	V	N	D	K	V	E	N
K	S	E	S	A	H	P	V	G	S	P	G	E	S	G	T	S	G	W	R
P	N	A	V	T	P	E	Q	—	—	L	T	T	S	R	P	S	K	E	A
A	S	S	A	G	S	T	R	N	K	D	P	T	R	R	P	E	L	E	A
P	R	G	G	A	V	P	R	K	P	V	S	E	M	P	M	E	R	D	R
<hr/>																			
F	L	M	T	F	L	A	S	*											
M	L	L	L	L	*														
P	G	A	S	G	G	S	S	G	D	P	D	G	F	F	N	S	K	V	A
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	V	A
—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	P	A
Q	Q	R	A	A	—	A	L	S	L	S	T	L	A	S	P	K	G	G	S
P	Q	H	T	T	—	T	L	S	L	S	T	L	A	T	P	K	R	S	G
S	F	G	R	G	G	S	L	G	L	G	G	G	G	G	M	G	P	R	E
H	P	V	Y	I	V	Q	E	M	P	P	Q	S	P	A	N	I	Y	Y	K
H	P	V	Y	I	V	Q	E	M	P	P	Q	S	P	A	N	I	Y	Y	K
H	P	V	Y	I	V	Q	D	G	P	P	Q	S	P	P	N	I	Y	Y	K

Fig. 1

8/17

E	V	R	V	L	H	S	I	G	H	S	A	A	P	R	L	F	P	L	A	
K	I	N	V	L	E	D	F	E	G	E	N	P	Q	V	P	K	L	E	K	
G	C	H	L	F	L	T	T	V	P	V	L	W	S	L	L	G	S	*		
S	L	E	P	A	D	D	T	V	R	E	S	A	E	P	S	R	G	E	N	
G	G	D	T	P	S	P	L	C	L	L	L	L	L	L	I	I	R			
D	N	T	V	K	M	A	T	Q	A	—	—	—	—	—	—	—	P	G	S	R
G	T	N	G	R	S	S	T	T	S	P	F	V	K	P	N	P	G	S	S	
G	A	A	H	S	L	E	P	G	K	E	N	L	P	G	D	P	T	S	<u>N</u>	

E	E	A	A	V	G	A	G	C	V	I	F	I	I	I	F	L	T		
E	F	A	G	I	A	S	G	C	I	I	F	I	V	I	I	I	T	L	V
V	A	G	A	A	G	G	L	A	L	L	L	G	V	A	G	A	G	G	

G	T	A	G	T	E	P	S	D	I	I	I	P	L	R	T	T	E	N	N
N	N	N	G	S	E	P	S	D	I	I	I	P	L	R	T	A	D	S	V
A	E	P	G	E	L	G	I	A	L	R	G	G	G	A	A	D	_	P	P

V	*																		
V	*																		
V	*																		

Fig. 1

9/17

W	T	V	L	L	L	P	L
S	I	S	G	T	S	P	K
A	A	Q	T	P	R	I	P
<b>E</b>	<b>L</b>	<b>R</b>	<b>I</b>	<b>E</b>	*		
G	S	L	G	D	S	D	G
T	D	_	G	N	S	A	G
<u>A</u>	T	S	R	G	A	E	G
V L L L K L R K							
V L L L K Y R R							
A M C W R R R R							
Y							
F							
F							

Fig. 1

10/17

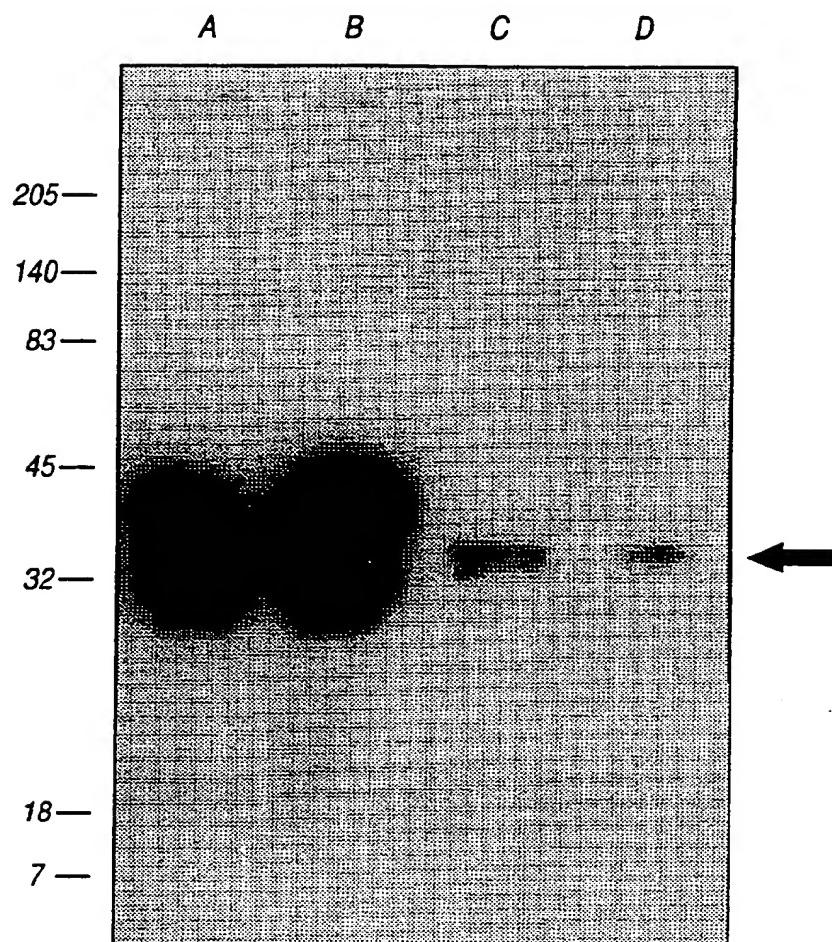
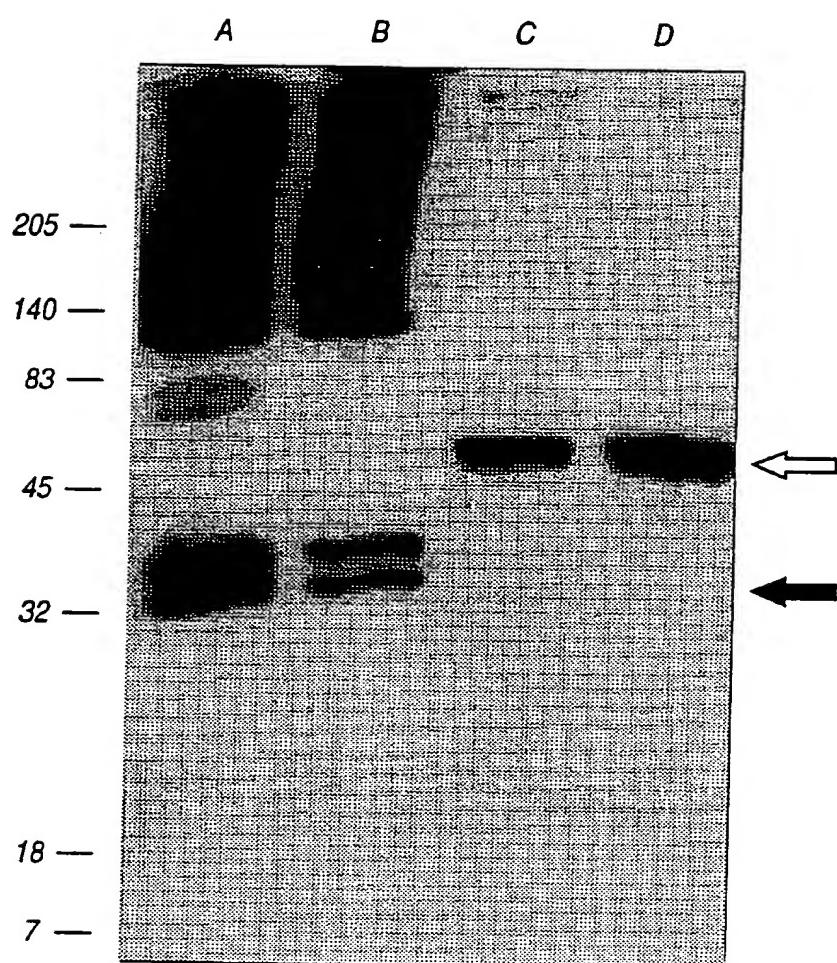


Fig. 2A

11/17



*Fig. 2B*

12/17

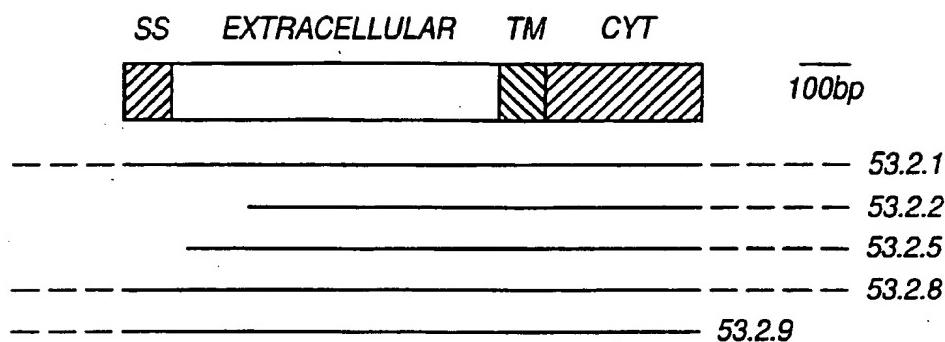
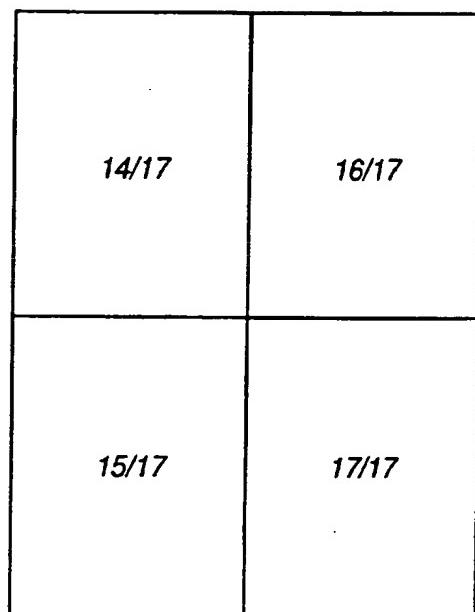


Fig.3

ASISSSSDYKDDDESRLNLEPVYW

Fig.5

13/17



*Fig.4*

SUBSTITUTE SHEET (Rule 26)

14/17

1	<u>ATGGGCCCCCCCATTCTGGGCCGGGGGC</u>
1	M G P P H S G P G G
61	<u>GTTTGGGGCTGGTGTCTGGCTCAGCCTG</u>
21	V L G L V S G L S L
121	AGGTTCCAGGCAGAGGGTGGTTATGTGCTG
41	R F Q A E G G Y V . L
181	CTCTGCCCGGGCCCGGCCTCCTGGCCCT
61	L C P R A R P P G P
241	CTGTACCTGGTAGGGGGTGCTCAGGGCCGA
81	L Y L V G G A Q G R
301	CTTCTCACTTGTGATGCCAGACCTGGAT
101	L L T C D R P D L D
361	AGCCCTAATCTCTGGGCCACGAGTCCGC
121	S P N L W G H E F R
421	TCGGATGGGACCCGGGAGGGCCTGGAGAGC
141	S D G T R E G L E S
481	ATGAAGGTGCTTCTCCGAGTGGACAAAGT
161	M K V L L R V G Q S

Fig. 4

15/17

541	GTGTCTGAAATGCCCATGGAAAGAGACCGA
181	V S E M P M E R D R
601	GAGAACCTGCCAGGTGACCCCACCAGCAAT
201	E N L P G D P T S N
661	CCCCCTCCCAGCATGCCT <u>GCAGTGGCTGGG</u>
221	P P P S M P A V A G
721	<u>GGCGTGGCAGGGGCTGGGGTGCCATGTGT</u>
241	G V A G A G G A M C
781	AGTCGCCACCCTGGTCCTGGCTCCTCGGG
261	S R H P G P G S F G
841	GGTGGGATGGGACCTCGGGAGGCTGAGCCT
281	G G M G P R E A E P
901	GGGCTGCAGATCCCCCTTCTGCCAC
301	G A A D P P F C P H
961	CCTGTGTATATCGTGCAGGATGGGCCCCC
321	P V Y I V Q D G P P
1021	tga
	*

Fig. 4

16/17

GTGCGAGTCGGGGCCCTCCTCCTACTGGGG  
V R V G A L L L L G

GAGCCTGTCTACTGGAACCTGGCGAATAAG  
E P V Y W N S A N K

TACCCTCAGATCGGGGACCGGCTAGACCTA  
Y P Q I G D R L D L

CACTCCTCTCCTAATTATGAGTTCTACAAG  
H S S P N Y E F Y K

CGCTGTGAGGCACCCCCCTGCCAAACCTC  
R C E A P P A P N L

CTCCGCTTCACCATCAAGTTCCAGGAGTAT  
L R F T I K F Q E Y

TCGCACCAACGATTACTACATCATTGCCACA  
S H H D Y Y I I A T

CTACAGGGAGGTGTGTGCCTAACCAGAGGC  
L Q G G V C L T R G

CCCCGAGGAGGGCTGTCCCCCGAAAACCT  
P R G G A V P R K P

Fig. 4

17/17

GGGGCAGCCCACAGCCTGGAGCCTGGGAAG  
G A A H S L E P G K

GCAACCTCCCCGGGTGCTGAAGGCCCTG  
A T S R G A E G P L

GCAGCAGGGGGCTGGCGCTGCTCTTGCTG  
A A G G L A L L L L

TGGCGGAGACGGCGGGCCAAGCCTTCGGAG  
W R R R R A K P S E

AGGGGAGGGTCTCTGGGCCTGGGGGGTGGAG  
R G G S L G L G G G

GGGGAGCTAGGGATAGCTCTGCAGGGTGGC  
G E L G I A L R G G

TATGAGAAGGTGAGTGGTGACTATGGGCAT  
Y E K V S G D Y G H

CAGAGCCCTCAAACATCTACTACAAGGTA  
Q S P P N I Y Y K V

Fig. 4

# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/AU 96/00460

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>		
Int Cl <sup>6</sup> : C12N 15/12; C07K 2/00, 4/12, 14/435; A61K 38/17		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) IPC6: Chemical Abstracts: keywords below.		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Medline, JAPIO: keywords below.		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) WPAT, JAPIO MEDLINE, CA: keywords: Lerk or RPTK; tyrosine kinase receptor or EPH or ERK or EHK or ECK or EFL.		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Oncogene, Volume 10 (2) issued January 1995, Kozlosky et al, "Ligands for the receptor tyrosine kinases hck and ehk: isolation of cDNA's encoding a family of proteins" pages 299-306. See entire document.	1-19
X	Cell, Volume 79, issued October 1994, Cheng and Flanagan "Identification of ELF-1 a developmentally expressed ligand for the Mek 4 and Sek receptor tyrosine kinases" pages 157-168. See entire document.	1-19
X	Science, volume 266, issued November 1994, Davies et al "Ligands for EPH - Related receptor tyrosine kinases that require membrane attachment or clustering for activity" pages 816-819. See entire document.	1-19
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex		
<ul style="list-style-type: none"> <li>* Special categories of cited documents:</li> <li>"A" document defining the general state of the art which is not considered to be of particular relevance</li> <li>"E" earlier document but published on or after the international filing date</li> <li>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</li> <li>"O" document referring to an oral disclosure, use, exhibition or other means</li> <li>"P" document published prior to the international filing date but later than the priority date claimed</li> </ul>		<ul style="list-style-type: none"> <li>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</li> <li>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</li> <li>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</li> <li>"&amp;" document member of the same patent family</li> </ul>
Date of the actual completion of the international search 12 September 1996		Date of mailing of the international search report <b>02.10.96</b>
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929		Authorized officer <b>KAREN AYERS</b> Telephone No.: (06) 283 2082

**INTERNATIONAL SEARCH REPORT**International Application No.  
**PCT/AU 96/00460**

<b>C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
<b>Category*</b>	<b>Citation of document, with indication, where appropriate, of the relevant passages</b>	<b>Relevant to claim No.</b>
PX	WO 96/10911 (Immunex Corp.) 18 April 1996, see entire document in particular example 1 and SEQ ID NO. 2	1-19
PX	WO 96/17925 (Immunex Corp) 13 June 1996, see entire document, in particular example 1 and SEQ ID No. 5.	1-19

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No.  
PCT/AU 96/00460

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member	
WO	9617925	AU	46393/96
WO	9610911	AU	38268/95

END OF ANNEX

**THIS PAGE BLANK (USPTO)**